Substrate conversion rates of $Mg^{2+}Ca^{2+}$-myofibrillar ATPases from eurythermal and stenothermal fish inhabiting different environmental temperatures

Species [Common name: habitat]	Environmental temperature (°C) [Range of habitat temperatures]	Substrate turnover number (moles ATP split · mole myosin^{-1} sec^{-1}) Assay at 0 °C	Assay at physiological temperature (°C)	Reference
Salvelinus fontinalis [brook trout: European rivers]	acclimated to +4	2.69±0.26	4.00±0.32 (4)	This study
	acclimated to +24	2.89±0.16	40.69±2.36 (24)	
Carassius auratus [common goldfish: European lakes]	acclimated to +1	1.40	1.45 (1)	3
	acclimated to +26	0.39	5.11 (26)	
Notothenia rossii [South Georgia cod: antarctic marine]	+2 [−1 to +4]	3.56	6.22 (4)	10
Gadus morhua [cod: North Sea]	+12 [+3 to +15]	1.02	8.96 (12)	13
Salmo trutta [brown trout: European lakes]	+15 [+5 to +24]	1.36	9.20 (15)	10
Abudefuf oxydon [neon reef perch: Indo-Pacific coral reefs]	+25 [+24 to +30]	0.15	4.76 (25)	10
Amphiprion sebae [Anenome Fish: Indo-Pacific coral reefs]	+25 [+24 to +30]	0.19	8.44 (25)	18
Tilapia nigra [Cichlid spp.: East African lakes]	+28 [+24 to +33]	0.25	6.67 (28)	13

Values are means of 6 or more preparations; SEM in each case < 15% of mean (see original references for further details). Substrate turnover numbers are calculated assuming a myosin content of 54% and a mol.wt of 240,000 daltons per enzyme active site (2 sites per myosin molecule)[16].

py ΔH^+) is comparable to that of a stenothermal species adapted to 8–10 °C (figure 2b).

It would appear that the properties of brook trout myofibrillar ATPase constitutes a compromise between the optimum kinetic forms for either the higher or the lower acclimation temperature.

The upper lethal limit for many salmoniids (22–24 °C) is much less than body temperatures routinely encountered by cyprinids in the natural habitats[17]. It is suggested that a combination of behavioural temperature regulation and a 'compromise' ATPase provides one alternative strategy for adaptation of locomotory function in eurythermal fish.

* Present address: Department of Biological Sciences, University of London, Goldsmiths College, Lewisham Way, London SE14 6NW (England).

1 The authors are grateful to the Wellcome Trust for financial support. - Correspondence should be addressed to I.A.J.

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Distribution of chromium in red kidney beans (*Phaseolus vulgaris* L.)

V. Ramachandran, T.J. D'Souza and K.B. Mistry

Biology and Agriculture Division, Bhabha Atomic Research Centre, Trombay, Bombay 400085 (India), 31 October 1980

Summary. Distribution of both Cr^{3+} and CrO_4^{2-} in bean shoots followed a markedly acropetal gradient. Chemical fractionation of radiochromium accumulated in the edible bean pods indicated the greatest association (70–75%) with ionic forms (extractable by weak mineral acids).

Chromium, which is present as a soil and water pollutant due to chromium wastes released from various industrial sources, and chromium-51, a gamma-emitting activation product released in controlled or accidental discharges from nuclear installations, could enter the human food-chain through rivers, groundwater and irrigated soil. Con-

flicting reports on the differential absorption and uptake of trivalent chromium ions (Cr^{3+}) and hexavalent chromium ions (CrO_4^{2-}) have appeared during the last few years[1,2]; but hardly any quantitative data are available on the distribution of the 2 forms of chromium in various plant organs and the metabolic fate of root-absorbed chromium in the edible parts of the plant. These aspects have been examined in the present investigation, using ^{51}Cr as a tracer for stable chromium.

Materials and methods. Red kidney beans (*Phaseolus vulgaris* L.) were germinated in quartz sand and then grown in 1 l of ^{51}Cr labelled nutrient solution. The nutrient solution contained K^+, 3.0; Ca^{2+}, 8.0; Mg^{2+}, 3.0; NO_3^-, 10.0; SO_4^{2-}, 3.0; $H_2PO_4^-$, 1.0; mequivalents/l together with micronutrients Fe, Mn, Cu, Zn, B and Mo. $^{51}CrCl_3$ and $Na_2{}^{51}CrO_4$, each having a sp. act. of 50 mCi/mg Cr, constituted separate treatments and were added at an activity level of 10 μCi ^{51}Cr/l (equivalent to 0.11 ng ^{51}Cr plus 200 ng stable Cr/l) to 5 replicate jars. The initial pH of the solution was adjusted to 6.0. A single bean plant was grown in each jar. The experiment was conducted in a growth room where the temperature was maintained at 23 ± 1 °C, the relative humidity 65 ± 2% and the plants were illuminated daily for 12-h periods at 1300 $\mu W \cdot cm^{-2}$ measured at 10 cm above the top of the jars. The transpiration losses from the solution were made up daily with distilled water. The plants were harvested and separated into different tissues when the bean pods (edible tissue/part of the plant) had fully developed (6 weeks after germination). The procedure for chemical fractionation of the bean pods has been described in detail in our earlier publications[3,4] and is indicated briefly under 'results and discussion'. The final samples were assayed by gamma-ray spectrometry[3,4] using the photopeak of 325 keV for quantitative estimation of ^{51}Cr.

Results and discussion. Data on the distribution of $^{51}Cr^{3+}$ and $^{51}CrO_4^{2-}$ in the various tissues of bean plants grown to pod formation are shown in table 1. Data indicate massive accumulation of both forms of chromium in the roots under conditions of nutrient culture experiments. Among the aerial tissues, the ^{51}Cr content was found to be the highest in the primary leaves and decreased with each succeeding trifoliate leaf up the stem from the base to the apex; very much reduced amounts were present in the stems and pods. The observed maximum concentration of ^{51}Cr in the oldest leaves with a progressive reduction in younger tissues is indicative of an acropetal gradient in the distribution of Cr in bean plants. These results also indicate a significant lack of redistribution of chromium in the bean plants as evidenced by the marked concentration gradient of chromium of more than 2 orders of magnitude between the oldest primary leaves and the pods. The distribution pattern of chromium was similar to that of manganese but differed from that of other transition elements like iron, cobalt and zinc which are more or less uniformly distributed in the plant[3].

Chemical characterization of chromium in the bean pods was undertaken to determine the metabolic fate of root-absorbed chromium. Pods (2 g) immediately after harvest were successively extracted as indicated in table 2. Data in table 2 indicate that about 11% of $^{51}Cr^{3+}$ was present in the ethanol fraction (lipids including lipophyllic pigments, free amino acids and amino sugars). The association of $^{51}Cr^{3+}$ was highest (74.9%) in the extracts of weak mineral acids which comprised ionic forms including salts of organic acids, phosphates, carbonates and some protein bound forms. Further 6.7% of $^{51}Cr^{3+}$ was present in pectates, proteins and polysaccharides (acetone plus soda fractions) and 7.8% in cellulose and lignin (residue). In the case of $^{51}CrO_4^{2-}$, 13.5% was in the ethanol fraction, 70.7% was in the acid fractions, 12.0% was associated with pectates, proteins and polysaccharides and 3.8% with cellulose and lignin. Neither $^{51}Cr^{3+}$ nor $^{51}CrO_4^{2-}$ was associated with the nucleic acid fraction. Previous studies on the distribution of chromium in the aerial parts of *Leptospermum scoparium*, a chromium accumulator plant growing on serpentine soils[5], and in the leaves of cauliflower plants grown in ^{51}Cr-labelled nutrient solution[6], have reported the predominant association of chromium with ionic complexes, notably the

Table 1. Distribution of ^{51}Cr in different tissues of bean plants. Age of plants: 42 days; duration of ^{51}Cr treatment: 32 days

Plant part	Radionuclide content (cpm/g dry wt)* $^{51}Cr^{3+}$	$^{51}CrO_4^{2-}$
Roots	235× 10^3	201× 10^3
Stem	70± 5	51± 16
Primary leaves	3710± 151	1871± 282
Trifoliate-1	516± 48	583± 137
Trifoliate-2	323± 59	436± 110
Trifoliate-3	189± 19	298± 84
Trifoliate-4	111± 4	139± 14
Axillary leaves + flowers	93± 24	181± 76
Pods	16± 4	10± 2

* Mean values ± SE.

Table 2. Fractionation of ^{51}Cr in the pods of bean plants. Age of plants: 44 days; duration of ^{51}Cr treatment: 35 days

Extractants	Fraction containing	Distribution of ^{51}Cr (% of quantity in pods) $^{51}Cr^{3+}$	$^{51}CrO_4^{2-}$
95% ethanol	Lipids including lipophyllic pigments, free amino acids and amino sugars	10.62 ± 0.06	13.47 ± 0.01
0.2 M HCl			
a) Supernatant	Ionic forms including salts of organic acids, phosphates, carbonates and some protein bound forms	69.66 ± 0.05	64.63 ± 0.03
b) Precipitate with acetone	Proteins and pectates	2.35 ± 0.01	6.42 ± 0.01
0.5 M $HClO_4$			
a) Supernatant	Remaining ionic forms	5.24 ± 0.15	6.04 ± 0.06
b) Precipitate with acetone	Nucleic acids	ND	ND
2 M NaOH	Remaining proteins and polysaccharides	4.27 ± 0.03	5.57 ± 0.02
Residue	Cellulose and lignin	7.86 ± 0.25	3.87 ± 0.23

The error term is the mean of triplicates. ND, not detected.

trioxalotochromate (Cr^{3+}) form. The present findings on the distribution of chromium in bean plants and in the major biochemical moieties present in bean pods are of environmental significance since they are indicative of the chemical forms in which chromium occurs in the edible parts of leguminous crops grown in contaminated soils.

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Arnaud's 'enigmatic little marks': an extension (type 6) to the manginuloid hyphae series of epiphyllous microfungi

R.T. Lange

Department of Botany, University of Adelaide, Box 498, G.P.O., Adelaide 5001 (South Australia), 13 November 1980

Summary. The use of manginuloid hyphae (a category of epiphyllous microfungal structures) in the interpretation of Tertiary vegetation palaeohabitats is discussed briefly. A new type (6) is added to the known series of types of present-day manginuloid hyphae. This type apparently is restricted to very wet, near-equatorial mid-montane rainforest and extends the basis for uniformitarian deductions about tertiary vegetational palaeohabitat.

Identical or closely similar kinds of distinctive microfungi can be found on both living and Tertiary fossil leaves[1-9]. The likelihood of finding certain kinds of microfungi by standardized short search in living vegetation of the Australian region is linked to particular habitats and vegetation-types[8,9]. These links are used to interpret the palaeohabitat and palaeovegetation-type of fossil leaf beds in the region via the fossil microfungi exhibited[7-9].

Mycological taxonomy has yet to deal with many of the species detected on living leaves by palaeontologists in their search for comparison material[8,9]. Where that is so, palaeobotanical work has to rely on ad hoc form-systematics of living as well as fossil forms, which is the case when epiphyllous microfungi exhibiting manginuloid hyphae are under consideration[9].

A special characteristic of manginuloid hyphae is the invisibility or evanescence of external walls, resulting in display of the pronounced septa as 'enigmatic little marks'[10] upon the host leaf. An ad hoc classification of present-day manginuloid hyphae devised to allow palaeontological progess with fossil forms culminates in type 5, of which *Vizella banksiae* Swart[11] is typical. In this paper a new present-day form extending the series (type 6) is reported.

Hyphae of both types 5 and 6 involve extremely short broad dark cells interspersed with long hyaline cells but in type 5 the 2 pronounced septa delineating short dark cells look similar and are close together but do not touch each other[11]. In type 6 they are dissimilar and do touch each other (figs 1, 2). Throughout type 6 hyphae, the short cell septum distal to the hyphal apex appears ordinary and transverse, but the septum proximal to the hyphal apex appears as a semicircle based on the misection of the transverse septum which projects on either side of the semicircle (fig. 2).

Mycological interpretation of such structure is difficult because external hyphal walls cannot be seen clearly. In these circumstances various interpretations are possible. If the hyphal outer wall is an invisible cylindrical tube, then the 2 septa appear to be arranged like a cup upside down in a saucer, so that the dark cell is totally enclosed within the hypha and its transverse septum is shared by three cells. If the hypha is flattened like a ribbon, some alternative interpretation may be correct.

A knowledge of type 6 further refines the available basis for uniformitarian interpretation of Australasian Tertiary vegetation habitats via fossil manginuloid hyphae, of which more discoveries are under investigation[12]. Type 5, which in

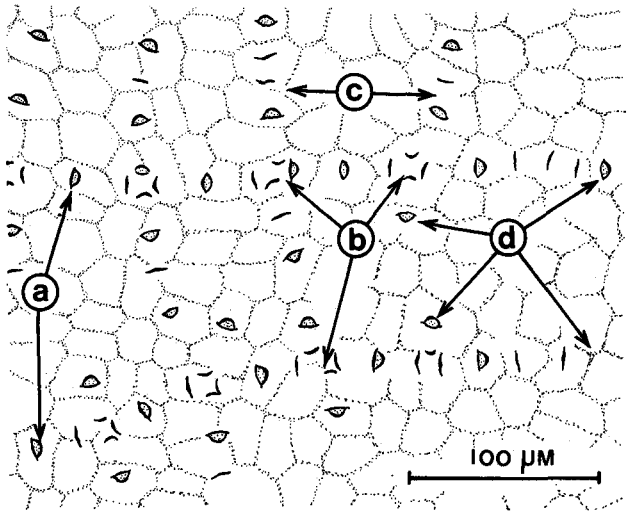

Figure 1. Drawing of hyphae at the periphery of a type 6 manginuloid mycelium: a, cells of main hyphae which run from left to right; b, cells bearing bilateral branch hyphae; c, cells of branch hyphae; d, hyphal tips of branch (left) and main (right) hyphae.

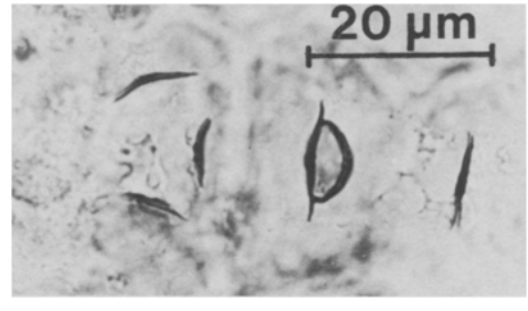

Figure 2. Oil immersion brightfield detail of type 6 manginuloid hypha showing branch cell (left), short cell (centre) and hyaline long cells.